(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 455 757 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
31.03.1999 Bulletin 1999/13

(21) Application number: **90913611.1**

(22) Date of filing: **03.08.1990**

(51) Int Cl.6: **C12M 3/06**, C12M 1/36, C12M 1/12, C12M 1/00

(86) International application number:
PCT/US90/04361

(87) International publication number:
WO 91/02049 (21.02.1991 Gazette 1991/05)

(54) **AN INTEGRATED CELL CULTURE-PROTEIN PURIFICATION SYSTEM FOR THE AUTOMATED PRODUCTION AND PURIFICATION OF CELL CULTURE PRODUCTS**

INTEGRIERTES ZELLKULTUR-PROTEINREINIGUNGSSYSTEM ZUR AUTOMATISIERTEN HERSTELLUNG UND REINIGUNG VON ZELLKULTURPRODUKTEN

SYSTEME INTEGRE DE CULTURE DE CELLULES-PURIFICATION DE PROTEINES POUR LA PRODUCTION ET LA PURIFICATION AUTOMATISEES DE PRODUITS DE CULTURE CELLULAIRE

(84) Designated Contracting States:
DE FR GB IT NL SE

(30) Priority: **04.08.1989 US 389606**

(43) Date of publication of application:
13.11.1991 Bulletin 1991/46

(73) Proprietors:
- **GRANDICS, Peter**
  Arcadia, CA 91077 (US)
- **SZATHMARY, Susan**
  Arcadia, CA 91077 (US)

(72) Inventors:
- **GRANDICS, Peter**
  Arcadia, CA 91077 (US)
- **SZATHMARY, Susan**
  Arcadia, CA 91077 (US)

(74) Representative: **Nicholls, Michael John et al**
J.A. KEMP & CO.
14, South Square
Gray's Inn
London WC1R 5LX (GB)

(56) References cited:
EP-A- 0 046 915
EP-A- 0 201 086
US-A- 3 883 393
US-A- 4 033 825
US-A- 4 490 290
US-A- 4 537 860

EP 0 455 757 B1

Printed by Jouve, 75001 PARIS (FR)

Description

Background

[0001] Complex proteins are increasingly used in research, diagnostics and therapeutics. Many of these proteins can only be produced in appropriate eucaryotic cells. With the advent of hybridoma technology and other progress in genetic engineering of eucaryotic cells, mammalian or yeast cell lines are becoming the method of choice for producing complex proteins on a large scale.

[0002] The secreted product needs to be purified from the cell culture medium. Most mammalian cells require serum which contains a diverse mixture of proteins, many of which are present at high concentrations. Even in serum-free media systems, numerous other proteins are secreted from the cells. For most of.the applications the final product has to meet high levels of purity and activity.

[0003] The successful production of these proteins depends largely on the development of fast and efficient methods of purification. Typically, the purification constitutes the major cost (up to 80% of the total cost) in these processes. The large scale use of these protein products is hindered because of the high cost.

[0004] There is an urgent need for processes to produce proteins in a simple and economical way. Significant cost reduction in the production of protein biologics coulds be realized if the purification would be integrated with cell culture into a fully automated system. In addition, the product quality is also expected to improve because the secreted protein is continuously removed from the culture medium in which the product is exposed to catabolic enzymes. Protein identity is an important issue for protein biotherapeutics, i.e. the final product should be free of degraded or other aberrant protein molecules. The integration requires that the presence of the purification unit in the cell culture system would not affect the conditions of cell culture. Therefore, highly-specific purification methods like affinity/immunoaffinity chromatography is needed to make the integration of cell culture and purification feasible for the continuous purification of secreted product.

[0005] Progress in cell culture technology has led to the development of membrane bioreactors for growing eucaryotic, such as mammalian cells within well-defined compartments. Cells grown inside low non-specific adsorption flat sheet or hollow fibre membranes in thin (200 to 400 um) layers are continuously perfused with nutrients and grow to cell densities previously unattainable by the stationary, stirred tank or airlift-type fermentors. Nutrient deprivation or shear sensitivity issues are minimised by this technology. This allows high cell viability in the bioreactor and minimises DNA contamination of the product. The microfiltration membrane eliminates the opportunity of bacterial contamination of the bioreactor. The cells are grown at tissue density with high production rates surpassing the production capacity of conventional bioreactors. After populating the available compartment space, the cells reach a growth-arrested state in which most of their energy is directed towards production. This configuration allows the highest production capacity per unit volume of bioreactor space.

[0006] US-A-4 537 860 discloses a reactor for maintaining cells in a growth-arrested state for secretion of cell products. Fresh nutrient is supplied by perfusion through low porosity tubes. Expended medium and cell product is withdrawn through high porosity tubes. Oxygenation is supplied via a membrane.

[0007] EP-A-0 201 086 discloses a system for cell culture and purification in accordance with the precharacterising portion of claim 1. It discloses a system in which culture medium may be drawn from a culture vessel, ultrafiltered and the liquid which does not pass through the filter is returned, the filtrate being removed.

[0008] Another important aspect of the integration is the availability of appropriate protein separation technologies. Current protein purification technologies require significant improvement in order to realise the potentials of the integration concept. A major obstacle is that the interaction of the cell culture medium with the protein separation material (chromatography resin) may change the composition of the medium which can be detrimental to the cells in culture. Chromatography media like ion exchange or hydrophobic matrices can drastically change the culture medium composition and thus are unsuitable for an integrated instrument if continuous removal of the product is desired. Biospecific, affinity separation is the only method offering the least interference with cell culture. However, current affinity technologies have serious shortcomings which have prevented them from being incorporated into an integrated system.

[0009] The integration of cell culture with continuous purification of secreted product without jeopardising the cell culture by introducing potentially toxic chemicals and bacterial/viral contamination necessitates the development of a stable, nontoxic, chemically inert, sterilizable activated affinity chromatography resin. Current activated affinity matrices cannot be incorporated into the integrated instrument because they do not meet the criteria of being chemically inert, nontoxic, stable, and sterilizable. The most commonly used coupling methods employ reactive electrophilic centers with leaving group displaced by the incoming nucleophilic ligand (protein/antibody). These displacement reactions frequently remain incomplete even after capping the unreacted sites and continue to release leaving groups, many of them are toxic to cells. Conversely, constituents of the culture medium may be covalently attached to the matrix. The affinity resin may leach other toxic molecules, like isocyanate from CNBr-activated matrices, for long periods of line. This is toxic to the cells in the bioreactor. The immobilization method may also increase the protease sensitivity of

immobilized protein (antibody) ligand, an issue which is a problem with the traditional coupling chemistries.

[0010] In affinity separations, proteins (antibodies) are frequently used as ligand. In the integrated system, the immunoaffinity chromatography resin must withstand the conditions of cell culture for long periods of time. The warm, highly-oxygenated environment of cell culture medium may diminish the activity of the immunoaffinity column. Many of the cultured mammalian cells, including hybridomas, secrete proteolytic enzymes which may degrade the immobilized antibody ligand. The same applies to dead cells spilling their content into the culture medium. The structure of the support and the method of immobilization also plays an important role in the protease sensitivity of immobilized antibody. The low concentration of secreted proteins in the cell culture medium may also complicate quantitative recovery of the product.

[0011] Current immunoaffinity technologies make process automation complicated because of the continuous loss of immunosorbent capacity. This is the result of ligand leaching and inactivation of immobilized antibody for reasons mentioned above. The total cycle life of the immunoadsorbent (5-30 cycles) is usually too short to make this technology suitable for the integration of bioreactor and purification for continuous product recovery. All these issues need to be addressed in order to make affinity chromatograply media compatible with mammalian cell culture.

[0012] According to the present invention, there is provided a cell culture and purification system for producing purified cell culture products, comprising: a cell culture sub-unit for culturing cells, whereby said cells can secrete said product into a culture fluid; a purification sub-unit linked to the cell culture sub-unit adapted to remove said product from said culture fluid; and means for circulating said culture fluid from said cell culture sub-unit to said purification sub-unit; characterised in that: the purification sub-unit comprises a stable, non-toxic and sterilizable activated resin as a solid-phase biospecific affinity medium for separating said product by specific affinity interaction; and in that said cell culture sub-unit, said purification sub-unit and said means for circulating are combined to form an integrated instrument for continuous automated production and purification of cell culture products in a sterile environment without removing said culture fluid from said instrument.

[0013] The invention will be further described by way of example only, with reference to the accompanying drawings, in which:-

[0014] Figure 1 is the flow diagram of the invention integrated cell culture/purification system.

[0015] Figure 2 is the accumulated production of purified monoclonal antibody in the integrated cell culture/purification system.

List of Reference Numerals

[0016]

2.	Cell culture unit/bioreactor
3.	Snap connectors
4.	Purification unit/chromatography cartridge
6.	Oxygenator
8.	Cell culture medium vessel
10.	Pump B
12.	Cell culture medium container
14.	Pump A
16.	Base container
18.	Acid container
20.	Compressed air source
22.	Carbon dioxide source
24,26,28,29.	Level sensors
30.	Gas flow control
32,34.	Injection ports
36.	Three-way valve
38,40.	Two-way valves
42.	Four-way valve
44,45,46,48,50.	Three-way valves
52.	pH Probe
53.	Temperature probe
54.	Dissolved oxygen probe
56.	Pump C
58.	Culture medium container
60.	Wash medium container

62.	Elution medium container
64.	Flow cell UV monitor
66	Waste fluid container
68	Product vessel
70	Gradient former
72, 74, 76, 78, 80	Level sensors
82, 84, 86, 88, 89	Three-way valves
90	Two-way valve
92, 94	Two-way air valves
96	Compressed air source
98, 100, 102, 103, 105, 112	Sterile filters
104	Pressure sensor
106	Bubble sensor
108	Controller
110	Fraction collector

Description of the Preferred Embodiment

[0017] Celle culture is a versatile technique for producing a variety of complex biomolecules including proteins. The protein of interest is produced and secreted by the cultured cells into the cell culture fluid of which the product is recovered, in many cases, by using complicated, multi-step purification procedures. This frequently results in significant product losses and an increased possibility of generating aberrant protein molecules. We have developed a new integrated instrument which unifies the formerly separate cell culture and protein purification into an integrated, automated operation which significantly reduces the manufacturing cost of high purity cell culture protein products. The product quality is also increased as a result of the integrated production of proteins. The integrated cell culture/purification instrument has two subunits, such as the bioreactor and chromatography subunits (Fig. 1).

[0018] In the subject invention, the cell culture unit is a hydrophilic hollow fiber bioreactor (Zymax, Microgon (Reg. Trade Mark). The bioreactor 2 consists of compact coaxial fibers in a cylindrical housing. The fibers are usually 0.2-1.0 mm in diameter with a pore size of 0.2 micron. The space within the fibers is called intro-capillary space (ICS) and the outside region is designated extra-capillary space (ECS). The cells are detained in ECS whereas ICS has nutrient or culture medium flowing through. The pore size of the fibers is small enough to contain the cells (approx. 10 microns in diameter), but allows exchange of nutrients and proteins across the membrane by diffusion.

[0019] The bioreactor environment needs to be carefully controlled because slight changes may lead to decreased productivity or cause cell death. The important parameters to be controlled are temperature, pH, dissolved oxygen and nutrient levels of the culture medium. Mammalian cells are very sensitive to chemical contaminants. A wide variety of substances even at ppm level could be highly toxic to the cells. Under the right culture conditions the cells remain active in the bioreactor for a couple of months or even longer.

[0020] To control bioreactor conditions, probes, such as the temperature probe 53, pH electrode 52 and dissolved oxygen probe 54 need to be included into the bioreactor loop and linked to the programable controller 108. A suitable controller may be a microprocessor controlled unit like the Proteus 2000 (Reg. Trade Mark) (Wheaton Scientific Instruments). The controller 108 receives information from the probes and makes appropriate adjustments in the above culture conditions. The pH is adjusted by autotitration of the culture medium from acid 18 or base 16 containers. The temperature is adjusted by warming the medium flask while the dissolved oxygen is changed by a gas flow controller 30 by increasing the air pressure on the oxygenator 6.

[0021] The oxygenator 6 (Microgon (Reg. Trade Mark)) is also of hollow fiber-type containing hydrophobic fibers with a pore size of 0.02 micron; 5% CO_2 in air mixture is passed through the oxygenator. The bioreactor loop contains the culture medium supply system which, in the simplest case, is a medium container changed periodically as the medium gets exhausted. In an automated fashion, feeding of cells is accomplished by continuous perfusion (feed and bleed system) by introducing fresh and withdrawing spent medium from the bioreactor loop at a preset rate. This is accomplished by using the reversible pump 14 (Pump A) which first withdraws spent medium from the medium vessel 12 and then replenishes it with fresh medium. The spent medium exits the system through the valve 45 and hollow fiber filter 103, 0.2 micron, into the drain. Alternatively, the spent medium may be recycled through valves 45, 46 onto the purification unit 4 for subsequent purification of the product.

[0022] The bioreactor 2 and affinity chromatography column 4 is enclosed into plug-in type cartridges which are presterilized and attached to the instrument through snap-in connectors 3. After each run, the cartridges are discarded. The rest of the instrument can be sterilized in place by using a chemical sterilant, such as 5% glutaraldehyde. After the specified time of sterilization, the glutaraldehyde is drained and the whole system is extensively washed with sterile, pyrogen-free deionized water (tissue culture grade) introduced through the connecting port of gradient former 70. The

bioreactor loop is then filled with culture medium and the bioreactor 2 is inoculated with the cells. The nutrients are delivered to the cells in the bioreactor 2 at a flow rate of 30-150 ml/min by using Pump B10.

[0023] The bioreactor loop contains aseptic injection ports 32, 34. Through port 32, either medium can be withdrawn from the bioreactor loop or compounds can be introduced into the cell culture medium. Through port 34, the bioreactor is inoculated with the cells to start operation. The four-way valve 42 and the three-way valves 48, 50 direct the culture medium on or off the purification unit 4. The purification unit 4 is not in operation before the cells populate the bioreactor. In the continuous mode of operation, after the sixth day, the controller 108 initiates the product recovery cycles.

[0024] The bioreactor was inoculated with $5x10^7$ hybridoma cells. The mouse x mouse hybridoma (HB 57) producing IgG1 monoclonal antibody was grown in RPM1 1640 medium supplemented with antibiotic solution and 10% iron-supplemented calf serum. This hybridoma is a low producer and requires a minimum of 10% serum for optimal growth and antibody production. The culture medium is kept at 37 C in the bioreactor loop. Cell density and viability were determined by using hemocytometer and Trypan blue staining.

[0025] The bioreactor loop interfaces with the purification/chromatography loop through the purification unit 4 which can be an affinity cartridge. The affinity cartridge is a polymeric cylindrical container closed with porous disks at the top and bottom and is filled with a fast flow activated affinity resin, Actigel-ALD Superflow (Reg. Trade Mark; patent pending) to which an antibody to the desired product is attached. Actigel-ALD Superflow (Sterogene Bioseparations, Inc.) is stable, nontoxic and sterilizable (autoclavable) activated resin. In this example, an affinity-purified, goat anti-mouse light chain-specific antibody is attached to the resin at a concentration of 10 mg/ml. The immunoaffinity resin has an extremely low content of leachables (< 0.1 ppm IgG) and is not toxic to mammalian cells. In the adsorption mode of operation, the culture medium is continuously circulated through the affinity cartridge 4 which adsorbs the monoclonal antibodies, secreted by the hybridomas in the bioreactor 2, from the culture medium. Periodically, the affinity cartridge 4 is taken off-line from the bioreactor loop by using the valves 42, 48 and 50 and the product recovery cycle is initated by the controller 108.

[0026] The separation loop is driven by Pump C 56 which delivers the wash-, elution-, and regeneration media (60; 62; 58) in the specified sequence. First, the residues of the tissue culture medium and nonspecifically adsorbed proteins are removed by extensive washing with the wash medium 60, 0.3 M NaCl. This step is followed by the elution of monoclonal antibodies from the affinity column 4 by using 1 bed volume of ActiSep Elution Medium 62 (Reg. Trade Mark; patent pending) over a period of 30 min. ActiSep (Sterogene Bioseparations, Inc.) is a nondenaturing elution medium allowing 100 or more cycles to be performed on immunoaffinity columns. The eluant retains the binding capacity of the immunoadsorbent during many cycles of operation as well as high bioactivity of the eluted product. Eluted protein peak is monitored by OD_{290} measurement in a flow cell UV photometer and the protein peak is integrated to quantitate eluted antibody. The product is collected into a 68 refrigerated storage bottle while the washes are collected into a separate 66 waste bottle. When all the elution medium is recovered from the affinity column, another wash with 0.3 M NaCl is initiated to remove traces of the eluant. This is followed by a wash with the 58 cell culture medium (RPMI 1640) to equilibrate the column for the subsequent product adsorption cycle.

[0027] The waste and product bottles 66, 68 are emptied by compressed air 96 introduced through sterile filter 100. The air flow is directed by the valves 92 and 94 into the respective bottles. The waste exits the system into the drain through sterile filter 98, 0.2 micron pore size, and two-way valve 90. The product exists the system through the sterile filter 102, 0.2 um pore size, into a collection flask from which it is collected by the operator.

[0028] The emptying of the waste and product vessels is initiated by the controller 108 receiving signals from the level sensors 78 and 80. The level sensors 72, 74, and 76 monitor fluid levels in the buffer vessels 56, 58, 60 and 62. The controller 108 issues warning signals to the operator to fill empty bottles. Until the buffer/medium bottles are replenished, no further product adsorption and elution cycles are initiated by the controller. The separation loop contains additional safety controls, such as pressure and bubble sensors 104, 106 to protect the purification unit 4 from pressure build-up or from running dry.

[0029] The accumulated antibody production of the system is shown in Fig. 2. After an approximately 1 week lag period, the antibody level has exceeded 10 ug/ml in the culture medium and continouous separation of the product has commended. Over a period of 60 days, an accumulated 800 mg of affinity-purified monoclonal antibody was recovered from the integrated system. The purity of antibody was tested by SDS-polyacrylamide gel electrophoresis. Single heavy and light chains were observed indicating a protein purity of approximately 99%. Importantly, no sign of degradation of antibody is found which underscores the significance of the integration concept, i.e., the continuous removal of the product for the culture fluid in which the antibody is exposed to proteolytic enzymes deriving from the serum and dead cells. Therefore, besides improving the economy of the process, the product quality is also improved.

[0030] If proteins other than antibodies need to be purified in a continuous fashion from the cell culture medium, the operator has to immobilize his antibody to the product to purification unit 4 which, in this case, contains Actigel-ALD Superflow activated support. The antibody mixed with the coupling reagent is injected into cartridge 4 through sterile port 32 and the reaction is allowed to take place for 6 h (Sterogene Bioseparations, Inc., Actigel-ALD Superflow Technical Bulletin). Unbound protein is removed by washing with wash medium 60 and then with cell culture medium 58 to

prepare the cartridge for the product adsorption cycle.

[0031] The major advantages of the continuous removal of the product from the cell culture medium are as follows:

1. Significant improvement in product quality. The immediate removal of the protein product from the cell culture minimizes the chances of product degradation.
2. Significant reduction in process development time. The cell culture process development can be minimized because there is no need to adapt the cells to low serum or defined media. Serum-containg culture medium can be used because the affinity purification of the product eliminates the concern as to the presence of serum proteins. The generic purification method, immunoaffinity chromatography significantly simplifies product recovery.
3. Significant reduction in the manufacturing cost of cell culture products. The cost of cell culture can be reduced by a factor of 5-10 because there is no need to use defined media or expensive fetal bovive serum. The specificity of immunoaffinity separation eliminates contaminants of bovine serum origin; thereofe there is no need for low IgG fetal serum. The cost of protein purification is also reduced by a factor of 10-15 because of the automation and long-term utilization of the immunoadsorbent.

[0032] Because of these advantages, continuous product recovery is strongly preferred. However, in certain cases when the characteristics of the product justifies it, discontinuous product recovery by other methods may be used. If the secreted product is resistant to proteolysis/degradation and the protein concentration in the cell culture medium is low, i.e. a low protein, defined medium is used, the product may be recovered from the spent culture medium in a discontinuous fashion as follows:

[0033] 1. The spent medium, withdrawn from the medium vessel 8 by reversible pump 14 (Pump A) is directed by three-way valves 45, 46 onto separation unit/chromatography cartridge 4 which may contain an ion exchange-, hydrophobic interaction(HIC)-, or affinity chromatography medium. In the event, if ion exchange chromotography is used, the column may contain e.g. a DEAE-type medium, equilibrated with 50 mM Tris, pH 8.0, delivered from vessel 62. Vessel 60 is filled with 50 mM Tris, pH 8.0, 1 M NaCl regeneration buffer. Vessel 58 contains a 2 mM Tris, pH 8.0 dilution buffer delivered by pump 56 (Pump C) to chromatography cartridge 4 along with the culture medium to lower the ionic strength of the cell culture medium. The appropriately diluted cell culture medium is applied to ion exchange cartridge 4 and washed with the equilibration buffer 62. The desired product is then elution by a gradient elution, generated by gradient former 70 from 0% (equilibration buffer) to 100% (end buffer, such as 50 mM Tris, pH 8.0, 0.3 M NaCl). The eluted product is measured at 280 nm in the flow cell UV, monitor 64. The protein concentration is calculated by integrating the elution peak. Through the three-way valve 89 and sterile filter 112, 0.2 micron pore size, eluted product exits the system into the fraction collector 110. The column is regenerated for the next adsorption cycle by washing with the regeneration buffer (vessel 60) and equilibration buffer (vessel 62).

[0034] 2. In the event if HIC is preferred for the purification of the product, the cartridge 4 contains an appropriate medium, such as phenyl-, octyl-, butyl-, hexyl, isopentyl-liganded or other HIC media. The HIC cartridge 4 is equilibrated with the binding salt by using gradient former 70. The cell culture medium is then applied as described for the ion exchange chromatography separations. The binding salt is applied through gradient former 70 at the appropriate ratio. Unbound materials are washed with the binding salt solution and then product is recovered by applying a low salt buffer or a reverse salt gradient onto the cartridge 4 through the gradient former 70. Through the three-way valve 89 and sterile filter 112, 0.2 micron pore size, eluted product exits the system into the fraction collector 110. The column is regenerated for the next adsorption cycle by washing with the regenerators buffer (vessel 60) and equilibration buffer (70 gradient former).

[0035] 3. In the discontinuous product recovery mode, affinity chromatography may also be used. For example, purification unit 4 may contain immobilized Protein A or Protein G capable of binding antibodies. The culture medium is applied to the affinity cartridge 4 in accord with paragraph 1. A wash fluid, e.g. 50 mM Tris, pH 8.0, 0.15 M NaCl is then applied from vessel 60 and directed to waste bottle 66. The lenght of the wash is determined by monitoring OD_{280} in the waste fluid. The bound antibody is then eluted by delivering one column volume of ActiSep Elution Medium from vessel 62 over a period of 30 min onto the cartridge 4. If pH gradient elution is desired, gradient former 70 can generate the required pH gradient profile. Through the three-way valve 89 and sterile filter 112, 0.2 micron pore size, eluted product exits the system into the fraction collector 110. The column is regenerated for the next adsorption cycle by washing with the equilibration buffer (60 vessel).

[0036] Besides operating as a cell culture/purification system, the instrument can also operate as a stand alone cell culture unit to culture and characterize cell lines. This is important if a new cell line need to be developed and culture conditions optimized for the production of a particular protein.

[0037] When the cell culture conditions are optimized, an optimal purification strategy can be developed for the product which may be a continuous immunoaffinity method or a discontinuous conventional, ion exchange or HIC purificalion or some other affinity methods. This decision is made based on the cell culture conditions and the sensitivity of the product to degradation as well as the intended use of the protein. For therapeutic applications where the product

identity is a major issue, continuous product purification is desirable as this method protects the product against degradation. If the product is more resistant to degradation and protein identity is of a lesser problem, discontinuous product purification may be suitable.

[0038] If purification of the secreted product is not desired, the separation loop can be utilized, independently from the bioreactor loop, for the purification of proteins like antibodies from biological fluids, such as serum or ascitic fluid. For the purification, ion exchange, HIC or affinity methods may be used following the description of paragraphs 1; 2 and 3. In general, the sample to be purified is applied to purification unit 4 from vessel 58. This is followed by the application of the wash fluid from vessel 60 and then elution is initiated either by applying a single eluant from vessel 62 or using gradient elution through gradient former 70.

[0039] Polishing purification of the product, obtained by continuous, immunoaffinity purification of secreted protein can also be accomplished as described in paragraphs 1, 2 and 3. These features allow the utilization of the instrument for the integrated production and purification of secreted product from cell culture with the built-in flexibility of applying a number of different purification strategies for product recovery with the objective of obtaining pure protein product while minimizing changes in product identity.

Claims

1. A cell culture and purification system for producing purified cell culture products, comprising: a cell culture sub-unit (2,8) for culturing cells, whereby said cells can secrete said product into a culture fluid; a purification sub-unit (4) linked to the cell culture sub-unit (2,8) adapted to remove said product from said culture fluid; and means (42-50) for circulating said culture fluid from said cell culture sub-unit (2,8) to said purification sub-unit (4); characterised in that: the purification sub-unit (4) comprises a stable, non-toxic and sterilizable activated resin as a solid-phase biospecific affinity medium for separating said product by specific affinity interaction; and in that said cell culture sub-unit (2,8), said purification sub-unit (4) and said means (42-50) for circulating are combined to form an integrated instrument for continuous automated production and purification of cell culture products in a sterile environment without removing said culture fluid from said instrument.

2. The system of claim 1, further including means (108,62) for discontinuing said circulation of cell culture fluid through said purification sub-unit (4) and eluting purified product from said purification sub-unit (4).

3. The system of claim 1, wherein said cells are separated from the purification sub-unit (4) by a membrane.

4. The system of claim 1, wherein said cell culture sub-unit (2,8) comprises a bioreactor sub-unit (2) for permitting contact between said cells and said culture fluid while preventing said cells from leaving said bioreactor sub-unit (2) when said culture fluid is circulated to said purification sub-unit (4).

5. The system of claim 4, wherein said bioreactor sub-unit (2) is of hollow fibre type and said cells are retained inside said hollow fibres.

6. The system of claim 1, wherein said cell culture sub-unit (2) further comprises an oxygenator (6) for supplying oxygen to said cells by oxygenating said culture fluid.

7. The system of claim 1, wherein said purification sub-unit (4) comprises a self-contained plug-in cartridge adapted for ready attachment to or removal from said instrument.

8. The system of claim 4 or 5, wherein said bioreactor sub-unit (2) comprises a self-contained plug-in type bioreactor cartridge adapted for easy attachment to or removal from said instrument.

9. The system of claim 4, 5, or 6, wherein said bioreactor sub-unit (2) comprises a thermostated culture medium vessel to maintain optimal temperature for cell culture.

10. The system of claim 4, 5, or 6, wherein said bioreactor sub-unit (2) comprises a gas flow regulator (20) to provide adequate oxygenation for the cell culture.

11. The system of claim 7, wherein said purification cartridge (4) comprises an activated medium for covalent attachment of proteins/ligands.

12. The system of claim 11, wherein said medium comprises aldehyde functional groups.

13. The system of claim 11, wherein said activated medium is sterilisable by autoclaving or sodium hydroxide.

14. The system of claim 11, wherein said activated medium is derivatised with non-proteinaceous molecules.

15. The system of claim 11, wherein said activated medium is derivatised with proteins.

16. The system of claim 11, wherein said activated medium is derivatised with antibodies.

17. The system of claim 11, wherein said activated medium is derivatised with light chain specific antibodies.

18. The system of claim 11, wherein said activated medium is derivatised with heavy chain specific antibodies.

19. The system of claim 11, wherein said activated medium is derivatised with antibodies at a concentration of 1 mg/ml to 10 mg/ml resin.

20. The system of claim 11, wherein said protein is coupled to said activated medium in said purification cartridge (4) in such a way that said protein is injected onto said cartridge (4) through a sterile port (34).

21. The system of claim 1, wherein said purification sub-unit (4) adsorbs secreted product from the cell culture medium.

22. The system of claim 21, wherein said secreted product is a protein.

23. The system of claim 22, wherein said secreted protein is a monoclonal antibody.

24. A method of recovering secreted proteins using the system of claim 22 or 23, wherein said purification sub-unit is a purification cartridge and said secreted proteins are recovered from said purification cartridge (4) by the steps of:

(a) first, extensively washing the cartridge (4) with a wash buffer to remove contaminating proteins;

(b) eluting the cartridge (4) with a mild elution medium to recover adsorbed protein;

(c) regenerating the cartridge (4) by consecutive washes with wash buffer and cell culture medium for the subsequent adsorption cycle.

25. A method according to claim 24, wherein the purified proteins are quantitated by ultraviolet spectrophotometry and subsequent integration of the protein peak.

26. The system of claim 21, wherein the instrument is further operable in a discontinuous mode in which adsorption of said secreted product is a discontinuous process, said product being a secreted protein.

27. A method of recovering secreted proteins by discontinuous adsorption using the system of claim 26, wherein said purification sub-unit (4) is a purification cartridge and said secreted proteins are recovered from said purification cartridge by a discontinuous adsorption process comprising the steps of:

(a) withdrawing spent cell culture medium from said bioreactor sub-unit (2) by using a pump;

(b) directing said spent medium onto said purification cartridge (4) comprising an adsorption medium;

(c) removing unbound cell culture medium constituents by a wash process;

(d) recovering bound product by elution from said purification cartridge (4).

28. The method of claim 27, wherein said purification cartridge (4) comprises an affinity chromatography medium.

29. The method of claim 27 or 28, wherein said product recovery process involves changing the ionic strength and/or the pH in the purification cartridge (4) to weaken the specific affinity interactions by which the product is bound to

the purification cartridge.

30. The system of claim 1, wherein said system is equipped with a refrigeration unit to maintain the bioactivity of perishable materials.

31. The system of claim 1, wherein said purified cell culture product is kept refrigerated.

32. The system of claim 1, wherein waste fluids exit the system through sterile filter ports.

33. The system of claim 1, wherein said instrument is constructed to be sterilizable by the chemical sterilant glutaraldehyde.

34. The system of claim 33, wherein said instrument is constructed to be sterilizable by glutaraldehyde at a concentration of 2 to 15% (v/v).

35. The system of claim 1, wherein said instrument is constructed to be sterilizable by autoclaving.

36. The system of claim 1, wherein said bioreactor and purification sub-units (2,4) comprise respective cartridges and said system is set up for the production run by attaching the bioreactor (2) and purification (4) cartridges to the sterile system.

37. The system of claim 36, wherein said bioreactor and purification cartridges (2,4) are constructed to be presterilizable before connection to the system.

38. The system of claim 7, 36 or 37, wherein said purification cartridge (4) comprises protein binding ligand.

39. The system of claim 38, wherein said protein binding ligand is an antibody.

40. The system of claim 38, wherein said protein binding ligand is an affinity ligand.

Patentansprüche

1. Ein Zellkultur- und Reinigungssystem zum Erzeugen von gereinigten Zellkulturprodukten, aufweisend: eine Zellkultur-Untereinheit (2, 8) zum Züchten von Zellen, wobei die genannten Zellen das genannte Produkt in ein Kulturfluid absondern können; eine Reinigungs-Untereinheit (4), die mit der Zellkultur-Untereinheit (2, 8) verbunden und dazu befähigt ist, das genannte Produkt aus dem genannten Kulturfluid zu entfernen; und eine Einrichtung (42-50) zum Umwälzen des genannten Kulturfluids von der genannten Zellkultur-Untereinheit (2, 8) zu der genannten Reinigungs-Untereinheit (4); **dadurch gekennzeichnet**, daß die Reinigungs-Untereinheit (4) ein stabiles, nicht-toxisches und sterilisierbares aktiviertes Harz als ein biospezifisches Fest-Phase-Affinitätsmedium zum Trennen des genannten Produkts durch eine spezifische Affinitäts-Wechselwirkung aufweist; und daß die genannte Zellkultur-Untereinheit (2, 8), die genannte Reinigungs-Untereinheit (4) und die genannte Einrichtung (42-50) zum Umwälzen kombiniert sind, um ein integriertes Gerät für eine kontinuierliche, automatisierte Herstellung und Reinigung von Zellkulturprodukten in einer sterilen Umgebung ohne ein Entfernen des genannten Kulturfluids aus dem genannten Gerät zu bilden.

2. Das System nach Anspruch 1, ferner aufweisend: eine Einrichtung (108, 62) zum Unterbrechen der genannten Umwälzung des Zellkulturfluids durch die genannte Reinigungs-Untereinheit (4) und zum Eluieren des gereinigten Produkts aus der genannten Reinigungs-Untereinheit (4).

3. Das System nach Anspruch 1, bei welchem die genannten Zellen von der Reinigungs-Untereinheit (4) durch eine Membran getrennt sind.

4. Das System nach Anspruch 1, bei welchem die genannte Zellkultur-Untereinheit (2, 8) eine Bioreaktor-Untereinheit (2) aufweist, um einen Kontakt zwischen den genannten Zellen und dem genannten Kulturfluid zu erlauben, wohingegen die genannten Zellen daran gehindert werden, die genannte Bioreaktor-Untereinheit (2) zu verlassen, wenn das genannte Kulturfluid zu der genannten Reinigungs-Untereinheit (4) umgewälzt wird.

5. Das System nach Anspruch 4, bei welchem die genannte Bioreaktor-Untereinheit (2) vom hohlen Fasertyp ist und die genannten Zellen innerhalb der genannten hohlen Fasern gehalten sind.

6. Das System nach Anspruch 1, bei welchem die genannte Zellkultur-Untereinheit (2) weiterhin einen Oxygenator (6) aufweist, um durch Sauerstoffanreicherung des genannten Kulturfluids den genannten Zellen Sauerstoff zuzuführen.

7. Das System nach Anspruch 1, bei welchem die genannte Reinigungs-Untereinheit (4) eine unabhängige Einschubpatrone aufweist, die zu einem leichten Anbringen an oder zu einem leichten Entfernen von dem genannten Gerät befähigt ist.

8. Das System nach Anspruch 4 oder 5, bei welchem die genannte Bioreaktor-Untereinheit (2) eine unabhängige Einschub-Bioreaktor-Patrone aufweist, die zu einem leichten Anbringen an oder zu einem leichten Entfernen von dem genannten Gerät befähigt ist.

9. Das System nach Anspruch 4, 5 oder 6, bei welchem die genannte Bioreaktor-Untereinheit (2) einen thermostatgeregelten Kulturmedium-Behälter aufweist, um die optimale Temperatur für die Zellkultur aufrecht zu erhalten.

10. Das System nach Anspruch 4, 5 oder 6, bei welchem die genannte Bioreaktor-Untereinheit (2) einen Gasstromregler (20) aufweist, um eine angemessene Sauerstoffanreicherung für die Zellkultur vorzusehen.

11. Das System nach Anspruch 7, bei welchem die genannte Reinigungs-Patrone (4) ein aktiviertes Medium für eine kovalente Hinzufügung von Proteinen/Liganden aufweist.

12. Das System nach Anspruch 11, bei welchem das genannte Medium funktionelle Aldehyd-Gruppen aufweist.

13. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium durch Autoklavbehandlung oder Natriumhydroxid sterilisierbar ist.

14. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit nicht-eiweißartigen Molekülen derivatisiert wird.

15. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit Proteinen derivatisiert wird.

16. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit Antikörpern derivatisiert wird.

17. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit spezifischen Leichtketten-Antikörpern derivatisiert wird.

18. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit spezifischen Schwerketten-Antikörpern derivatisiert wird.

19. Das System nach Anspruch 11, bei welchem das genannte aktivierte Medium mit Antikörpern bei einer Konzentration von 1 mg/ml bis zu 10 mg/ml Harz derivatisiert wird.

20. Das System nach Anspruch 11, bei welchem das genannte Protein mit dem genannten aktivierten Medium in der genannten Reinigungs-Patrone (4) auf eine solche Art und Weise gekoppelt wird, daß das genannte Protein in die genannte Patrone (4) durch eine sterile Öffnung (34) injiziert wird.

21. Das System nach Anspruch 1, bei welchem die genannte Reinigungs-Untereinheit (4) ein abgesondertes Produkt aus dem Zellkulturmedium adsorbiert.

22. Das System nach Anspruch 21, bei welchem das genannte abgesonderte Produkt ein Protein ist.

23. Das System nach Anspruch 22, bei welchem das genannte abgesonderte Protein ein monoklonaler Antikörper ist.

24. Ein Verfahren zum Wiedergewinnen von abgesonderten Proteinen unter Verwendung des Systems nach Anspruch 22 oder 23, wobei die genannte Reinigungs-Untereinheit eine Reinigungs-Patrone ist und die genannten abge-

sonderten Proteine aus der genannten Reinigungs-Patrone (4) durch die folgenden Schritte wiedergewonnen werden;

(a) ein erstes, extensives Waschen der Patrone (4) mit einer Waschpuffersubstanz, um kontaminierende Proteine zu entfernen;

(b) Eluieren der Patrone (4) mit einem milden Eluierungsmedium, um ein adsorbiertes Protein wiederzugewinnen;

(c) Regenerieren der Patrone (4) durch aufeinanderfolgende Waschungen mit Waschpuffersubstanz und Zellkulturmedium für den darauffolgenden Adsorptionszyklus.

25. Ein Verfahren nach Anspruch 24, bei welchem die gereinigten Proteine durch Ultraviolett-Spektrophotometrie und darauffolgende Integration der Proteinspitze quantitiert werden.

26. Das System nach Anspruch 21, bei welchem das Gerät weiterhin gemäß einem diskontinuierlichen Modus betriebsfähig ist, bei welchem die Adsorption des genannten abgesonderten Produkts ein diskontinuierlicher Prozeß ist, wobei das genannte Produkt ein abgesondertes Protein ist.

27. Ein Verfahren zum Wiedergewinnen von abgesonderten Proteinen durch diskontinuierliche Adsorption unter Verwendung des Systems nach Anspruch 26, wobei die Reinigungs-Untereinheit (4) eine Reinigungs-Patrone ist und die genannten abgesonderten Proteine aus der genannten Reinigungs-Patrone durch einen diskontinuierlichen Adsorptionsprozeß wiedergewonnen werden, welcher die folgenden Schritte aufweist:

(a) Entnehmen des verbrauchten Zellkulturmediums aus der genannten Bioreaktor-Untereinheit (2) unter Verwendung einer Pumpe;

(b) Leiten des genannten verbrauchten Mediums zu der genannten Reinigungs-Patrone (4), welche ein Adsorptionsmedium aufweist;

(c) Entfernen von nicht gebundenen Zellkulturmedium-Bestandteilen durch einen Waschprozeß;

(d) Wiedergewinnen des gebundenen Produkts durch Eulieren aus der genannten Reinigungs-Patrone (4).

28. Das Verfahren nach Anspruch 27, bei welchem die genannte Reinigungs-Patrone (4) ein Affinitäts-Chromatografie-Medium aufweist.

29. Das Verfahren nach Anspruch 27 oder 28, bei welchem der genannte Produktwiedergewinnnngsprozeß ein Ändern der Ionenstärke und/oder des pH in der Reinigungs-Patrone (4) involviert, um die spezifischen Affinitäts-Wechselwirkungen abzuschwächen, durch welche das Produkt an die Reinigungs-Patrone gebunden wird.

30. Das System nach Anspruch 1, bei welchem das genannte System mit einer Kühleinheit ausgerüstet ist, um die Bioaktivität von verderblichen Materialien aufrecht zu erhalten.

31. Das System nach Anspruch 1, bei welchem das genannte gereinigte Zellkulturprodukt gekühlt gehalten ist.

32. Das System nach Anspruch 1, bei welchem Abfallfluide das System durch sterile Filteröffnungen verlassen.

33. Das System nach Anspruch 1, bei welchem das genannte Gerät so ausgebildet ist, um durch das chemische Sterilisierungsmittel Glutaraldehyd sterilisierbar zu sein.

34. Das System nach Anspruch 33, bei welchem das genannte Gerät so ausgebildet ist, um durch Glutaraldehyd bei einer Konzentration von 2 bis 15% (v/v) sterilisierbar zu sein.

35. Das System nach Anspruch 1, bei welchem das genannte Gerät so ausgebildet ist, um durch Autoklavbehandlung sterilisierbar zu sein.

36. Das System nach Anspruch 1, bei welchem die genannte Bioreaktor-Untereinheit (2) und die genannte Reinigungs-

Untereinheit (4) jeweilige Patronen aufweisen und das genannte System für den Produktionsablauf durch Anbringen der Bioreaktor-Patrone (2) und der Reinigungs-Patrone (4) an dem sterilen System eingerichtet ist.

37. Das System nach Anspruch 36, bei welchem die genannte Bioreaktor-Patrone (2) und die genannte Reinigungs-Patrone (4) so ausgebildet sind, um vor der Verbindung mit dem System vorsterilisierbar zu sein.

38. Das System nach Anspruch 7, 36 oder 37, bei welchem die genannte Reinigungs-Patrone (4) einen Proteinbindungsligand aufweist.

39. Das System nach Anspruch 38, bei welchem der genannte Proteinbindungsligand ein Antikörper ist.

40. Das System nach Anspruch 38, bei welchem der genannte Proteinbindungsligand ein Affinitätsligand ist.

Revendications

1. Système de culture et de purification de cellules permettant de produire des produits purifiés de culture cellulaire, comprenant - des unités secondaires de culture cellulaire (2, 8) pour cultiver des cellules, lesdites cellules pouvant sécréter ainsi ledit produit dans un liquide de culture ;

   - une sous-unité de purification (4) reliée aux sous-unités de culture cellulaire (2, 8), adaptée à éliminer ledit produit dudit liquide de culture ; et
   - des moyens (42, 50) pour faire circuler ledit liquide de culture depuis lesdites sous-unités de culture cellulaire (2, 8) vers ladite sous-unité de purification (4) ;

   lequel système est caractérisé par le fait que la sous-unité de purification (4) comprend une résine activée stable, non-toxique et stérilisable comme phase solide d'affinité biospécifique pour séparer ledit produit par interaction spécifique de type affinité, et par le fait que lesdites sous-unités de culture cellulaire (2, 8), ladite sous-unité de purification (4) et lesdits moyens (42, 50) pour faire circuler sont combinés pour former un instrument intégré permettant une production et une purification automatisées et en continu des produits de culture cellulaire dans un environnement stérile sans qu'il soit nécessaire de retirer ledit liquide de culture dudit instrument.

2. Système selon la revendication 1, comprenant en outre des moyens (108, 62) pour interrompre ladite circulation du liquide de culture cellulaire à travers ladite sous-unité de purification (4) et pour éluer ledit produit purifié de ladite sous-unité de purification (4).

3. Système selon la revendication 1, dans lequel lesdites cellules sont séparées de la sous-unité de purification (4) par une membrane.

4. Système selon la revendication 1, dans lequel lesdites sous-unités de culture cellulaire (2, 8) comprennent une sous-unité de bioréacteur (2) permettant le contact entre lesdites cellules et ledit liquide de culture tout en empêchant lesdites cellules de quitter ladite sous-unité de bioréacteur (2) lorsqu'on fait circuler ledit liquide de culture vers ladite sous-unité de purification (4).

5. Système selon la revendication 4, dans lequel ladite sous-unité de bioréacteur (2) est de type à fibres creuses et lesdites cellules sont retenues à l'intérieur desdites fibres creuses.

6. Système selon la revendication 1, dans lequel ladite sous-unité de culture cellulaire (2) comprend en outre un dispositif d'oxygénation (6) pour alimenter en oxygène lesdites cellules en oxygénant ledit liquide de culture.

7. Système selon la revendication 1, dans lequel ladite sous-unité de purification (4) comprend une cartouche enfichable autonome pouvant facilement être fixée audit instrument et retirée de celui-ci.

8. Système selon la revendication 4 ou 5, dans lequel ladite sous-unité de bioréacteur (2) comprend une cartouche de bioréacteur de type enfichable autonome pouvant facilement être fixée audit instrument et retirée de celui-ci.

9. Système selon la revendication 4, 5 ou 6, dans lequel ladite sous-unité de bioréacteur (2) comprend un récipient de milieu de culture thermostaté permettant de maintenir une température optimale pour la culture cellulaire.

10. Système selon la revendication 4, 5 ou 6, dans lequel ladite sous-unité de bioréacteur (2) comprend un dispositif de réglage du débit gazeux (20) permettant une oxygénation adéquate de la culture cellulaire.

11. Système selon la revendication 7, dans lequel ladite cartouche de purification (4) comprend une phase activée pour une fixation covalente de protéines/ligands.

12. Système selon la revendication 11, dans lequel ladite phase comprend des groupes fonctionnels aldéhyde.

13. Système selon la revendication 11, dans lequel ladite phase activée peut être stérilisée à l'autoclave ou avec de l'hydroxyde de sodium.

14. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des molécules non-protéiniques.

15. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des protéines.

16. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des anticorps.

17. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des anticorps spécifiques de chaînes légères.

18. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des anticorps spécifiques de chaînes lourdes.

19. Système selon la revendication 11, dans lequel ladite phase activée est modifiée par des anticorps à raison de 1 mg/ml de résine à 10 mg/ml de résine.

20. Système selon la revendication 11, dans lequel ladite protéine est couplée à ladite phase activée dans ladite cartouche de purification (4) de telle manière que ladite protéine est injectée dans ladite cartouche (4) à travers un orifice stérile (34).

21. Système selon la revendication 1, dans lequel ladite sous-unité de purification (4) adsorbe le produit sécrété du milieu de culture cellulaire.

22. Système selon la revendication 21, dans lequel ledit produit sécrété est une protéine.

23. Système selon la revendication 22, dans lequel ladite protéine sécrétée est un anticorps monoclonal.

24. Procédé de récupération de protéines sécrétées utilisant le système de la revendication 22 ou 23, dans lequel ladite sous-unité de purification est une cartouche de purification et lesdites protéines sécrétées sont récupérées à partir de ladite cartouche de purification (4) par les étapes consistant à :

(a) tout d'abord, laver abondamment la cartouche (4) avec un tampon de lavage pour en éliminer les protéines contaminantes ;
(b) éluer la cartouche (4) avec un milieu d'élution doux pour récupérer la protéine adsorbée ;
(c) régénérer la cartouche (4) en la lavant successivement avec un tampon de lavage puis avec le milieu de culture cellulaire en préparation du cycle d'adsorption suivant.

25. Procédé selon la revendication 24, dans lequel les protéines purifiées sont dosées par spectrophotométrie UV suivie de l'intégration du pic de protéine.

26. Système selon la revendication 21, dans lequel l'instrument peut en outre fonctionner selon un mode discontinu où l'adsorption dudit produit sécrété est un processus discontinu, ledit produit étant une protéine sécrétée.

27. Procédé de récupération de protéines sécrétées par adsorption discontinue utilisant le système de la revendication 26, dans lequel ladite sous-unité de purification (4) est une cartouche de purification et lesdites protéines sécrétées sont récupérées de ladite cartouche de purification par un procédé d'adsorption discontinu comprenant les étapes consistant à :

(a) retirer le milieu de culture cellulaire usé de ladite sous-unité de bioréacteur (2) à l'aide d'une pompe ;
(b) faire passer ledit milieu usé sur ladite cartouche de purification (4) comprenant une phase d'adsorption ;
(c) récupérer, par lavage, les constituants du milieu de culture cellulaire non liés ;
(d) récupérer le produit lié par élution à partir de ladite cartouche de purification (4).

28. Procédé selon la revendication 27, dans lequel ladite cartouche de purification (4) comprend une phase de chromatographie d'affinité.

29. Procédé selon la revendication 27 ou 28, dans lequel ledit procédé de récupération de produit implique le changement de la force ionique et/ou du pH dans la cartouche de purification (4) pour affaiblir les interactions d'affinité spécifique au moyen desquelles le produit est lié à la cartouche de purification.

30. Système selon la revendication 1, dans lequel ledit système est muni d'une unité de réfrigération permettant de conserver l'activité biologique des matières périssables.

31. Système selon la revendication 1, dans lequel ledit produit de culture cellulaire purifié est maintenu réfrigéré.

32. Système selon la revendication 1, dans lequel les déchets liquides sortent du système à travers des orifices filtrants stériles.

33. Système selon la revendication 1, dans lequel ledit instrument est construit de manière à pouvoir être stérilisé par un agent de stérilisation chimique de type glutaraldéhyde.

34. Système selon la revendication 33, dans lequel ledit instrument est construit de manière à pouvoir être stérilisé par du glutaraldéhyde à une concentration de 2 à 15 % (en volume).

35. Système selon la revendication 1, dans lequel ledit instrument est construit de manière à pouvoir être stérilisé à l'autoclave.

36. Système selon la revendication 1, dans lequel lesdites sous-unités de bioréacteur et de purification (2, 4) comprennent chacune une cartouche et ledit système est préparé pour la production par fixation des cartouches de bioréacteur (2) et de purification (4) au système stérile.

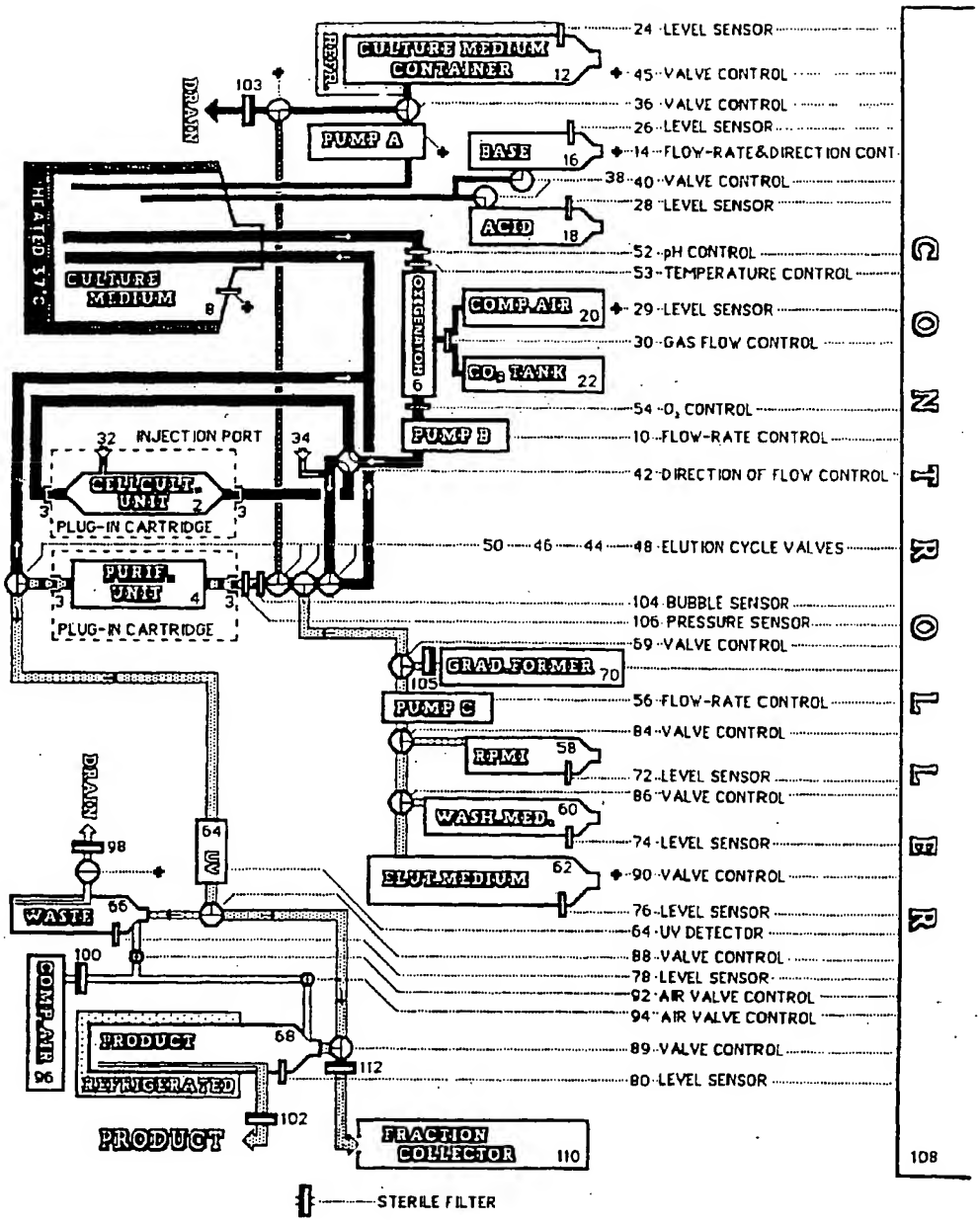
37. Système selon la revendication 36, dans lequel lesdites cartouches de bioréacteur et de purification (2, 4) sont construites de manière à pouvoir être stérilisées avant la connexion au système.

38. Système selon la revendication 7, 36 ou 37, dans lequel ladite cartouche de purification (4) comprend un ligand de fixation de protéines.

39. Système selon la revendication 38, dans lequel ledit ligand de fixation de protéines est un anticorps.

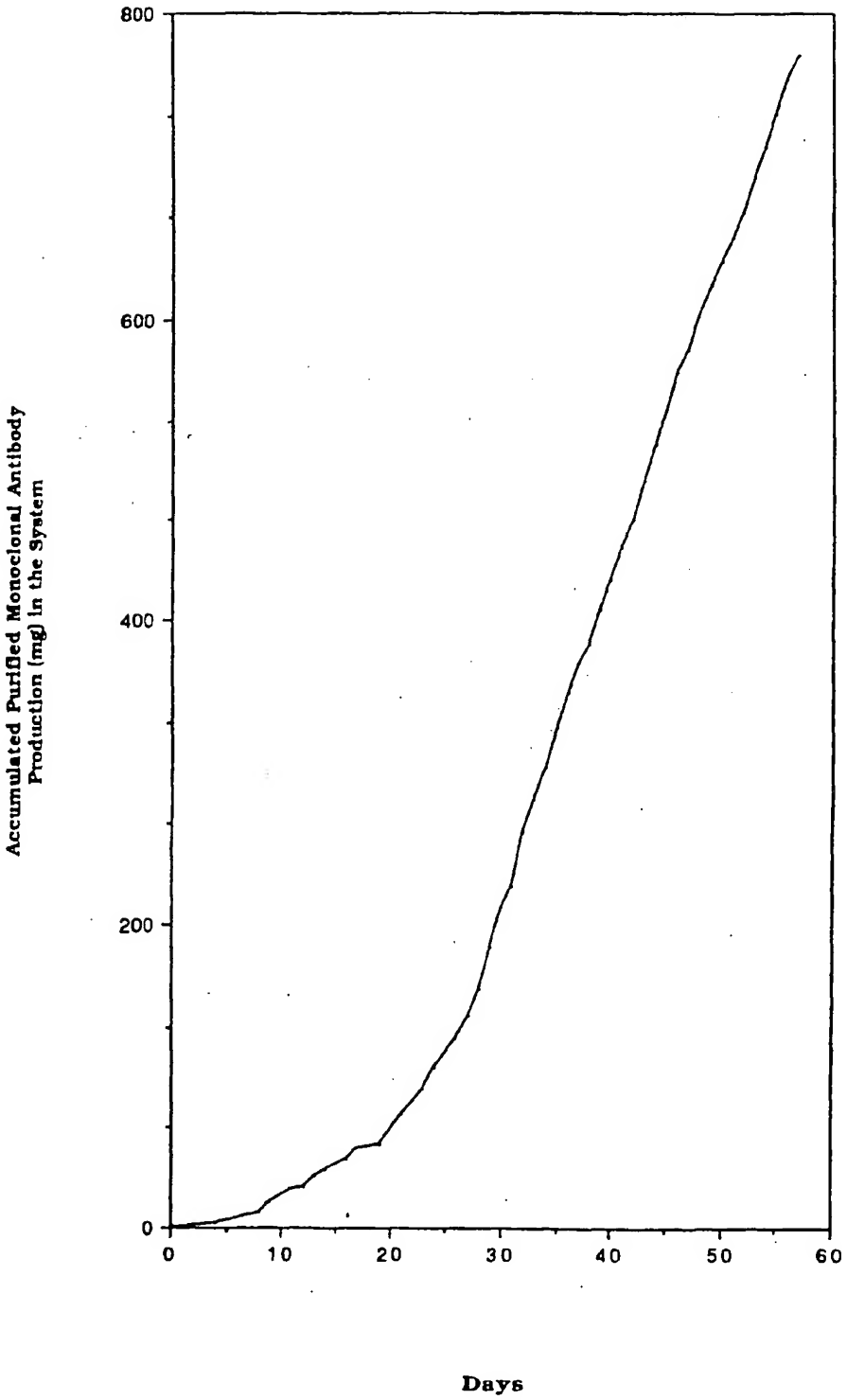
40. Système selon la revendication 38, dans lequel ledit ligand de fixation de protéines est un ligand d'affinité.

Figure 1



The Integrated Cell Culture Purification System

Figure 2


800
600
400
200
0
0
10
20
30
40
50
60
Accumulated Purified Monoclonal Antibody Production (mg) In the System
Days